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Biological Activity and Chemical Composition of the Essential Oil from Jamaican *Hyptis verticillata* Jacq.

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The chemical composition of the essential oil obtained by hydrodistillation from the aerial parts of *Hyptis verticillata* Jacq. was elucidated by a combination of GC and GC-MS analyses. The oil was dominated by the sesquiterpenoids cadina-4,10(15)-dien-3-one (15.1%) (**1**) and aromadendr-1(10)-en-9-one (squamulosone) (30.7%) (**2**). The oil exhibited chemosterilant activities against the cattle tick, *Boophilus microplus* Canest., and toxic action against adult *Cylas formicarius elegantulus* Summer, the most destructive pest of sweet potato (*Ipomoea* species).

KEYWORDS: ***Hyptis verticillata*; Labiatae; essential oil; sesquiterpene; *Boophilus microplus*; *Cylas formicarius elegantulus*; GC-MS; GC**

INTRODUCTION

The genus *Hyptis* (Labiatae) consists of over 400 species mainly found in the tropical Americas (*1*), and many plants from this genus are widely used in traditional medicine for the treatment of various illnesses. They have been found to possess tumorigenic, antifertility, antimicrobial, mycotoxic, and phytotoxic activities (*2*) as well as acaricidal, insecticidal, and nematicidal properties (*3*). Most of these plants are highly aromatic and hence of interest for investigations of their essential oils. The plant *Hyptis verticillata*, commonly called "John Charles" in Jamaica, has found widespread use in folk medicine. It is recognized for its astringency and is utilized in baths for skin diseases such as eczema, psoriasis, scabies, and athlete's foot (*4*). In Jamaica, the plant is a favorite cold medicine and is utilized alone on with other plants such as *Solanum torvum* (*5*).

Phytochemical investigations of *H. verticillata* have revealed the presence of the sesquiterpenes cadina-4,10(15)-dien-3-one (**1**) and aromadendr-1(10)-en-9-one (squamulosone) (**2**) (**Figures 1** and **2**) (*6*,*7*) as well as several lignans and triterpenes (*8*, *9*). Previous analyses of the volatile oils of this plant have indicated the possibility of different chemotypes. A plant sample obtained from Freiburg, Germany, revealed the presence of α-pinene (65.2%), *β*-pinene (8.5%), and thymol (1.6%) as the major components (*10*); however, the volatile oil from a Cuban plant was dominated by cadina-4,10(15)-dien-3-one (14.8%) and isocaryophyllene epoxide (14.4%) (*11*).

Here we report the chemical composition of the essential oils from a Jamaican variety of *H. verticillata* as well as its insect growth regulatory and insecticidal properties against adult female ticks (*Boophilus microplus*) and both sexes of the sweet potato weevil *Cylas formicarius elegantulus*, respectively. *B. microplus* is of considerable economic importance because of its ability to transmit disease-causing organisms in cattle (*12*). *C. formicarius elegantulus* is often considered to be the most

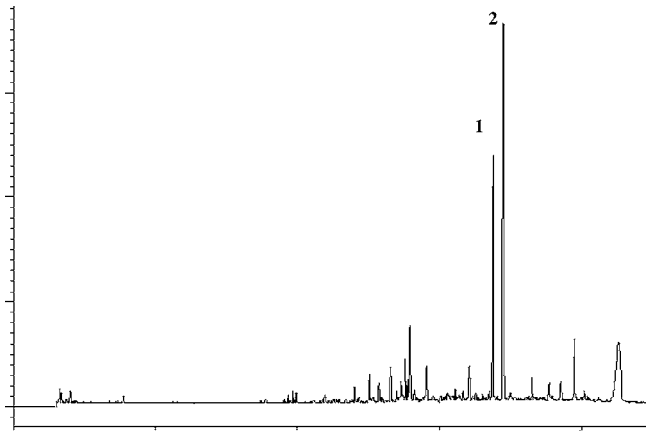

Figure 1. GC chromatogram from the analysis of the essential oils of *H. verticillata*.

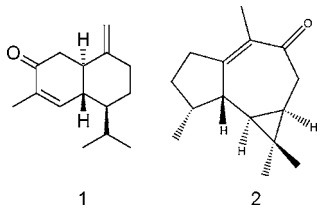

Figure 2. Structures of cadina-4,10(15)-dien-3-one (**1**) and aromadendr-1(10)-en-9-one (**2**) isolated from *H. verticillata*.

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serious pest of sweet potato, with reports of losses ranging from 5 to 97% in areas where the weevil occurs (*13*). A symptom of infestation by the weevil is yellowing of the vines, but a heavy infestation is usually necessary before this is apparent. Thus, incipient problems are easily overlooked, and damage is not apparent until tubers are harvested. The principal damage is caused by the mining of the tubers by larvae in addition to facilitating the entry of soilborne pathogens. Even low levels of feeding induce the production of toxic sesquiterpenes in the roots, which impart a bitter taste (*14*).

MATERIALS AND METHODS

Plant Material. The plant *H. verticillata* was collected in January 2001 along the banks of the Hope River in August Town, St. Andrew, Jamaica. An identified voucher specimen documenting this collection has been deposited in the herbarium of the Department of Life Sciences, University of the West Indies, Mona (accession no. 33483).

Isolation of the Essential Oils. The aerial parts of the green *H. verticillata* (398 g) were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. Distillation was carried out in triplicate. The essential oils obtained were dried over anhydrous sodium sulfate to consistently yield clear yellow oils in 0.15% yields (w/w).

Analytical Conditions. The chemical composition of the essential oils was analyzed by GC-MS using a Varian Saturn 2200 instrument interfaced to a Varian CP-3800 gas chromatograph. The gas chromatograph was equipped with a WCOT CP-SIL 8 fused-silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness); helium was used as the carrier gas with a flow rate of 1 mL/min. The temperature program employed was 80 °C (5 min) to 200 °C (5 min) at a rate of 3 °C/min, with injection and detector temperatures maintained at 250 and 220 °C, respectively. The mass spectral data were obtained on a Saturn 2000 ion trap mass spectrometer with an ionization energy of 70 eV and a mass range of 30−450 amu.

Calculation of Kovats Retention Indices (RI). The oil was spiked with a standard mixture of homologous *n*-alkane series (C_9−C_{25}) and analyzed by GC under the above-mentioned conditions. Retention indices were directly obtained by the application of the Kovats procedure (*15*).

Component Identification. The components of the essential oils were identified by comparison of their mass spectral data with those of the NIST 98 library or with authentic compounds and were confirmed by comparison of their Kovats retention indices with data published in the literature (*16*−*19*).

Insecticidal Assay. Two-week-old adult *C. formicarius elegantulus* (Summer) weighing 50 ± 2.5 mg each were used for bioassay. Insects were cultured on sweet potato tubers (*Ipomoea* species) in the laboratory at 25 ± 2 °C and 65−68% relative humidity (RH). A 25% (v/v, 0.05 mL in 0.2 mL of acetone) stock solution was prepared for the oils. From the above, 1.0, 2.0, 3.0, and 4.0 μL were topically applied to the abdomen of 20 adult *C. formicarius* in two replicates of 10 each using a Hamilton microapplicator. Twenty insects, each treated with 4.0 μL of acetone only, served as the controls. The number of dead insects was recorded at 24 and 48 h after treatment. Dimethoate was used as the positive control, and LD_{50} (concentration of dimethoate or *H. verticillata* oil required for killing 50% of test insect) was determined.

Acaricidal Assay. The assay was performed on fully engorged adult female *B. microplus* (Canest.) weighing 200 ± 10.0 mg within 4 h of collection from the local abattoir.

From the stock solution of the essential oil prepared for the insecticidal assay above, 2.0, 4.0, 6.0, and 8.0 μL aliquots (i.e., 1.0, 2.0, 3.0, and 4.0 μL of oil) were topically applied to the dorsa of the adult *B. microplus*. Twenty ticks (in two replicates of 10) were used for each bioassay. Treated ticks were kept in Petri dishes at room temperature (27 ± 2 °C) and 55−60% RH. The control experiment involved 20 ticks, which were treated with acetone (8.0 μL) under identical conditions. Dimethoate and eugenol were used as the positive controls. Mortality was recorded at 96 h after treatment. Dead ticks were removed and the survivors allowed to oviposit eggs. The percentage inhibition of oviposition (IO) was calculated by using the reduction in the weight of the eggs oviposited by the treated and control ticks during 21 days of the post-treatment period. From the data obtained an IOD_{50} value was determined (concentration of *H. verticillata* oil required for inhibiting oviposition by 50%), which was compared to dimethoate.

Oviposited eggs, after 21 days, were weighed and pooled. The viability of the eggs at each concentration was determined by placing them in test tubes plugged with cotton wool and incubating them for 6 weeks at 27 ± 2 °C and 50−60% RH. During incubation, the cotton was moistened every second day with distilled water. The ratio of hatched/unhatched eggs was determined by removing the hatched larvae, which had aggregated at the top of the test tube, and by examining the residue remaining at the bottom of the tube. Samples consisting of hatched and unhatched eggs were taken from each tube and examined under the microscope at a magnification of ×100. The ratio of hatched egg shells/unhatched eggs was used to establish the extent of inhibition of hatching according to Abbot's formula (*20*).

All mortality and inhibition of oviposition data were transformed to probit analysis before the calculation of LD_{50} and IOD_{50}.

RESULTS AND DISCUSSION

Chemical Composition. Steam distillation of fresh aerial parts of *H. verticillata* gave a strong odorous yellow oil in 0.15% yield. The very low leaf oil yield appears to be characteristic of the *Hyptis* genus (*21*−*25*). Analysis by capillary gas chromatography (GC) revealed that the oil was a complex mixture (**Figure 1**). The oil was resolved into 37 components, and 33 compounds were identified by comparison with retention indices, authentic samples when available, and the NIST mass spectral data library (**Table 1**). The oil was dominated by oxygenated sequiterpenes. The major components were the sesquiterpenoids cadina-4,10(15)-dien-3-one (**1**) (15.1%) and aromadendr-1(10)-en-9-one (squamulosone) (**2**) (30.7%), with minor components of viridiflorol (4.3%) and spathulenol (2.2%). Successive analysis of the oil gave very reproducible results, and all area data given are the average of three consecutive GC analyses. Cadina-4,10(15)-dien-3-one (**1**) had been previously reported in a Cuban variety of *H. verticillata* as major component; however, there has been no report of aromadendr-1(10)-en-9-one (**2**) in *Hyptis* oils. This suggests the Jamaican variety of *H. verticillata* may be the same chemotype as the Cuban plant; however, both plants differed from the German variety, in which monoterpenes were the major components (*10*).

Biological Studies. The oil was found to be an effective insecticidal agent against *C. formicarius* with a 48 h LD_{50} value of 0.4 μL/g insect compared with an LD_{50} of 0.13 μL/g of insect for a dimethoate concentration of 2 μL/g of insect at 48 h (**Table 2**). Data presented in **Table 3** indicate that the oil disrupted the oviposition and hatching of *B. microplus* eggs; however, it was not very lethal to the adult ticks (**Table 3**). Thus, doses of 1.0, 2.0, 3.0, and 4.0 μL/g of tick body weight inhibited oviposition (and hatching) by 43.75% (30.50%), 65.00% (50.20%), 79.87 (83%), and 87.20% (90%), respectively. The IOD_{50} was 1.5 μL/g of tick body weight. Eugenol, a known acaricidal agent in *Pimenta dioica*, gave an IOD_{50} value of 0.9 μL/g of body weight for *B. microplus*, and the commercial pesticide dimethoate gave an IOD_{50} of 1.7 μL/g of tick body weight (*26*). Acetone used as a solvent/negative control was totally inactive.

The acaricidal and insecticidal activities of the oil could be due to the synergistic effect of the major components cadina-4,10(15)-dien-3-one (**1**) and squamulosone (**2**), which both exhibited activities at levels of milligrams per gram of insect body weight (*6*, *7*). The oil, however, produced enhanced biological activity with significant results at levels of microliters per gram of insect body weight. The importance of this observation is that using the oil offers an added advantage

Table 1. Percentage Composition of the Essential Oil from the Aerial Parts of *H. verticillata*

compound[a]	RI	area %	ID[b]
1-ethylbutyl hydroperoxide	965	1.3	GC-MS
1-octen-3-ol	987	0.6	GC-MS, RI
linalool	1102	0.4	GC-MS, RI
α-ylangene	1368	0.2	GC-MS, RI
α-copaene	1375	0.4	GC-MS, RI
β-bourbonene	1382	0.7	GC-MS, RI
β-elemene	1389	0.5	GC-MS, RI
β-cubebene	1434	tr[c]	GC-MS, RI
longifolene	1437	0.4	GC-MS, RI
germacrene D	1461	tr	GC-MS, RI
dihydroaromadendrene	1472	tr	GC-MS
viridiflorene	1487	0.8	GC-MS, RI
epi-bicyclosesquiphellandrene	1494	tr	GC-MS, RI
γ-cadinene	1513	1.5	GC-MS, RI
δ-cadinene	1530	1.1	GC-MS, RI
di-*epi*-cedrene-1-oxide	1551	1.9	GC-MS
selina-3,7(11)-diene	1562	0.7	GC-MS, RI
ledene alcohol	1570	1.2	GC-MS
spathulenol	1577	2.2	GC-MS, RI
cedrene epoxide	1582	1.3	GC-MS, RI
viridiflorol	1586	4.4	GC-MS, RI
β-copaen-4-α-ol	1594	0.6	GC-MS, RI
cubenol	1615	1.6	GC-MS, RI
α-cadinol	1656	0.4	GC-MS, RI
3-oxo-β-ionone	1685	0.6	GC-MS
guaiol acetate	1735	0.6	GC-MS, RI
cadina-4,10(15)-dien-3-one	1743	15.1	GC-MS, GC-Co
aromadendr-1(10)-en-9-one	1764	30.7	GC-MS, RI, GC-Co
6S-2,3,8,8-tetramethyltricyclo-[5.2.2.0(1,6)]undec-2-ene	1821	1.2	GC-MS
4,6-diisopropylidene-8,8-dimethylbicyclo[5.1.0]-octan-2-one	1883	1.0	GC-MS
6-(1-hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1*H*-naphthalen-2-one	1909	3.1	GC-MS
methyl hexadecanoate	1930	0.6	GC-MS, RI
hexadecyl acetate	2004	12.0	GC-MS, RI
total		**87.1**	

[a] Elution order on CPSIL 8 capillary column. [b] GC-MS, tentative identification by gas chromatography–mass spectrometry; GC-Co, coelution with authentic sample; RI, retention index in DB-5. [c] Trace amounts (<0.1%).

Table 2. Toxic Action of Essential Oils against *C. formicarius elegantulus*[a]

dose (μL/g of insect body wt)	% mortality after 24 h	% mortality after 48 h
H. verticillata essential oils		
0.1	0.0	15
0.2	20.0	35.0
0.5	40	60
1.0	50.0	80.0
dimethoate		
0.1	10	30
0.2	30	60
0.5	50	90
1.0	80	100
control (4 μL of acetone)	0.0	0.0

[a] $N = 20$ in two replicates of 10 each.

because it is readily obtainable by simple hydrodistillation as opposed to the isolation of cadina-4,10(15)-dien-3-one (**1**) or squamulosone (**2**) via extensive chromatography.

The essential oil of *H. verticillata*, due to the plant's widespread occurrence, could provide an alternative as a natural product-based pesticide in the control of *B. microplus* and *C. formicarius*. It can also find application in the postharvest treatment of sweet potato tubers being placed into storage to prevent reinfestation from nearby fields.

Table 3. Effects of Oils on the Survivability of Fully Engorged Adult Female *B. microplus*[a]

dose (μL/g of tick body wt)	% mortality at 96 h	mean egg wt (mg) ± SE	% IO (after 21 days)	% IH
H. verticillata essential oils				
1.0	10	45.50 ± 3.5	43.75	30.5
2.0	25	28.32 ± 2.3	65.00	50.2
3.0	30	16.2 ± 4.0	79.87	83
4.0	45	10.3 ± 3.6	87.20	90
eugenol				
0.2	0.0	65 ± 2.0	14.0	30
0.5	20.0	50 ± 1.0	34.2	40.5
2.0	35.0	32 ± 0.8	57.89	50.6
3.0	80.0	20 ± 1.4	73.68	80.3
dimethoate				
0.2	0.0	75 ± 1.3	1.33	35.2
0.5	15.0	50.4 ± 4.0	33.68	50.6
1.0	30.0	35.9 ± 3.1	58.1	80.4
2.0	50.0	25.8 ± 4.5	66.1	
control (8 μL of acetone)	0	80.50 ± 2.3	0.0	0.0

[a] IO, inhibition of oviposition; IH, inhibition of hatching; SE, standard error.

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